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Twenty fetal sheep were exposed to impulses with peak levels of 169 dB SPL (pSPL). Auditory evoked potentials and behavioral state were recorded from the fetuses before and after impulse exposures. In the uterus of pregnant sheep, the pSPL varied as a function of fetal head location. When the fetal head was against the abdominal wall, peak levels were within 2 dB of airborne levels. When the fetal head was deep within the uterus, the peak amplitude decreased by more than 20 dB. Data from ten fetuses exposed at 117 days gestational age (dGA) and from 10 fetuses exposed at 127 dGA revealed slight elevations in post-exposure auditory brainstem response thresholds for low-frequency eliciting stimuli. Scanning electron microscopy revealed damage to inner and outer hair cells located in the middle and apical turns of the cochlea. Cochleae of fetuses exposed at 117 dGA showed greater damage than cochleae of fetuses exposed at 127 dGA. Recordings of behavioral state indicated disruption of normal cycling during and shortly after the exposure, however, individual variation was noted. The major finding is that impulse noise exposure produced significant damage to sensory hair cells located in the apical region of the inner ear in fetal sheep.

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INTRODUCTION

Subjects

This research addressed the effects of impulse noise exposures on the hearing, inner ear histology and sleep-state of fetuses. Fetal sheep were used to evaluate the effects of exposures delivered to the flank of pregnant animals. Two groups, comprised of ten fetuses each, included auditorily immature animals at 117 days gestational age (dGA) (term is 145 days) and auditorily mature ones at 127 dGA. Comparisons of evoked potentials, electrocortical activity and heart rate before and after exposure to 20 impulses with average peak levels of 169.9 dB were made to determine deleterious effects on the fetus. Scanning electron microscopy was used to evaluate histological changes within the cochleae.

Purpose

Knowledge of the transmission of exogenous sounds into the uterus and the effects that these sounds have on fetal hearing is incomplete. Safe maternal exposure levels have yet to be determined. The purposes of this study were to measure the transmission of impulse noises into the uterine environment and to evaluate the effects of exposures delivered to the flank of pregnant sheep on the hearing, inner ear histology and sleep-state of the fetus.

The following hypotheses were addressed: 1) low-frequency components of impulse noises will be transmitted into the uterus with little loss in energy, whereas the high-frequency components will be greatly attenuated; 2) impulse noise exposures will produce elevations in fetal auditory evoked potential thresholds that are dependent upon the frequency content of the eliciting stimulus and the age at which the exposure occurred; 3) behavioral state will be temporarily disrupted by an exposure to impulse noises; and 4) histology of inner ear tissue will reveal significant hair cell damage in the middle and apical turn of the fetal cochlea and the damage will be greater when the exposure occurs earlier in gestation.

Scope of Research

Externally generated steady-state sound is transmitted to the fetal head more efficiently for low-frequency sounds than for higher frequency sounds (Gerhardt, et al. 1990). This pattern of sound attenuation is similar for humans and sheep (Querleu et al., 1988; Richards, et al., 1992; Gerhardt et al., 1990). Calculations of sound attenuation produced by the tissues and fluids associated with pregnancy from human and animal studies have been based on microphone (hydrophone) measurements in the uterus compared to measurements in air near the abdominal wall. Transmission of impulse noise to the fetal head has important implications for women employed in the military, law enforcement and in other occupations where women are near impulses or impacts.

Auditory evoked responses (auditory brainstem response [ABR] and amplitude modulation following response [AMFR]) and scanning electron microscopy of inner ear tissue are useful tools in the assessment of auditory changes following noise exposures. Based on experience with auditory changes produced by steady-state noise exposures, it was hypothesized that animals exposed to impulse noise at an early gestational age (117 days) would not demonstrate immediate post-exposure threshold shifts. However, early noise-exposed fetuses would show poorer evoked potential thresholds, particularly for low frequencies, than fetuses

exposed later (127 dGA). It was expected that sensory cell loss would be apparent in the middle and apical region of the inner ear. Animals exposed to noise at 127 days would reveal an immediate threshold shift, which would probably recover to near normal levels after the exposure. Histopathologic procedures were expected to show modest hair cell loss.

The effects of exposure to impulse noises were evaluated through assessment of fetal behavioral state (sleep-state). Fetal sleep-state is an important event that relates directly to fetal brain growth and development. Behavioral state was evaluated before, during and immediately after exposure to impulses.

Background of Previous Research

The relation between exposures to intense sound and decreases in hearing sensitivity of adult male workers was first reported in the early 18th century. Since then, noise-induced hearing loss (NIHL) has been widely studied. The permanent and handicapping effects of intense noise on adult hearing have been well-documented. Recently, attention has shifted to the possibility of NIHL during fetal life (Gerhardt, 1990).

Significant numbers of American working women of childbearing age are noise exposed. The Committee on Hearing, Bioacoustics, and Biomechanics (CHABA, 1982), attempting to protect fetal hearing, suggested that pregnant women avoid noise exposures greater than 90 dB(A). Other investigators believed that these recommendations could needlessly exclude women from the work force (Niemtzow, 1993). There is a paucity of evidence to support either conviction. Two retrospective studies (Lalande et al., 1986; Daniel and Laciak, 1982) found increased risk of hearing loss in children with occupationally noise-exposed mothers. But these studies have been criticized for methodological shortcomings (Niemtzow, 1993; Henderson et al., 1993).

Findings from experimental animals have been paradoxical. Dunn et al. (1981) exposed pregnant sheep to intense steady-state noise for 4 hours a day, 5 days a week for several weeks. Thirty to forty days after the lambs were born, ABR thresholds were normal. The ABR, a far-field recording of a bioelectric response to sound from the auditory mechanism, is a common clinical and research hearing assessment tool. In contrast to the Dunn study, Griffiths et al. (1994) measured the ABR from fetal sheep *in utero* before and after a single 16-hour, broadband noise exposure (100 Hz to 10 kHz) at 120 dB sound pressure level. The investigators found significant changes in ABR thresholds and latencies immediately following noise exposure, although these changes were temporary. In a related study (Gerhardt et al., In Press), the fetal sheep ABR was recorded over a 23-day period following a similar noise exposure delivered at 113 days gestation (gestation for sheep is 145 days). No immediate changes in ABR thresholds were found, but thresholds for the noise-exposed group were significantly higher than for an age-matched, nonexposed group after 2 weeks or more. Cook et al. (1981) demonstrated ABR Wave IV latency differences between guinea pigs exposed to textile noise during the last trimester of gestation and a non-exposed control group.

In most instances, postnatal noise exposures occur in air. Prenatally, externally generated sounds must pass from air to the fluid medium of the uterus in order to reach the fetus. The intrauterine sound environment is dominated by frequencies below 0.5 kHz (Gerhardt et al., 1990). Externally generated sound transmission to the fetal head is more efficient for low

frequency, steady-state sounds than for higher frequency, steady-state sounds. Low-frequency sound pressure penetrate the uterus with little reduction in level and higher frequencies are reduced by about 20 dB. Transmission of impulse noise to the fetal head has not been measured, although it would be expected to follow the same pattern.

BODY OF THE FINAL REPORT

Methods, Assumptions and Procedures

During sterile surgery, the instrumentation for chronic recording of the evoked potentials was implanted in two groups of sheep, either at a gestational age of 115 days or 125 days. The fetus was exteriorized and the fetal head prepared for evoked potential and behavioral state recordings. A hydrophone was sutured near the fetal head in some preparations. The purpose of the hydrophone was to record acoustic levels in the intrauterine environment during the impulse noise exposure.

Two days after surgery, the ewe was placed in a sound-treated booth and fetal evoked potential thresholds were assessed using tone bursts and clicks. Behavioral state and heart rate were assessed for at least one hour before exposure in the older fetuses. After pre-exposure testing, ewes were exposed to 20 impulses produced by a shock tube. A second hydrophone, connected to one channel of a spectrum analyzer and positioned close to the maternal flank, recorded the pressure-time history generated by the shock tube. A simultaneous recording (channel 2) from the hydrophone *in utero* was obtained. Measurements from the two hydrophones were used to calculate transmission characteristics from air to the fetal head. The post-exposure evoked potentials were followed for 20 days in fetuses exposed at 117 days gestational age and for 10 days in the fetuses exposed at 127 days gestational age. At 137 days gestational age, the ewes and fetuses were sacrificed and cochleae removed and prepared for scanning electron microscopy.

Measurements of heart rate, electrocorticogram and electrooculography were simultaneously recorded on strip-chart paper and FM magnetic tape. These measurements were obtained one hour before exposure to 20 impulses, during exposure and for one-hour post exposure. The analog signals from the magnetic tape were subjected to spectral analyses in order to evaluate changes in behavioral state and heart rate responses produced by the stimulus.

We assumed that the impulses would be affected by transmission through maternal tissues and fluids, resulting in a reduction of peak sound pressure and a reduction in high-frequency spectral energy. We further postulated that fetal evoked potential thresholds would be elevated for low-frequency stimuli following exposure to impulses and scanning electron microscopy would reveal hair cell loss concentrated in the middle and apical turns. In addition, behavioral state cycling would be disrupted during and for a period of time immediately after the exposures.

Results and Discussion

Impulse Transmission into the Uterus. Impulses recorded in air and within the uterus of pregnant ewes averaged 169.9 and 162.6 dB peak sound pressure level (pSPL), respectively. The standard deviations were 0.92 dB in air and 5.94 dB in the uterus. Casual inspection of the pSPL recorded from the uterus revealed considerable variation. Figure 1 illustrates the difference in

pSPL among the animals. The solid bars represent the average of 20 impulses recorded in air from each animal and the cross-hatched bars represent the average of 20 impulses recorded in the uterus for each animal.

Figure 2 includes representative waveforms of an impulse recorded with one hydrophone in air and a second hydrophone sutured near the pinna of the fetus *in utero*. In this figure, the hydrophone in the uterus was located very close to the flank of the ewe that was nearest the shock tube. The morphology of these two waveforms is similar and the peak levels differ by only 3 dB (169 dB pSPL compared to 166 dB pSPL in the uterus). In other recordings from the uterus, there were marked differences in the morphology. Figure 3 includes recordings of an impulse recorded in air and the same impulse recorded with a hydrophone positioned deep within the uterus. In this figure, pSPL differed by 22 dB.

Waveforms, sampled for 100 ms, were processed through one-third octave-band filters from 100 to 10,000 Hz. The amplitude in each band was averaged across 10 animals. The averages were converted to decibels (re: 20 μPa) and plotted in Figure 4. Note that high-frequency sound pressure was attenuated by the tissues and fluids associated with pregnancy, findings similar to those reported in pregnant sheep and humans from earlier studies (Gerhardt, et al., 1990; Peters, et al., 1993). Low-frequency pressures within the uterus (below about 160 Hz) were similar to pressure levels recorded in air. Because of the high variability from *in utero* recordings, we completed a separate study, reported below, on the effects of intraabdominal location on impulse characteristics.

Effect of Intraabdominal Location on Impulse Characteristics. Impulse noise includes all forms of high-intensity short-duration sounds, i.e., from common industrial impacts to intense blast waves associated with military operations. Impulse durations vary from microseconds for small arms fire to hundreds of milliseconds for a sonic boom. Intensities for those signals range from less than 100 dB to over 185 dB peak sound pressure level (pSPL).

The signatures of the impulses also may vary. Impact noise, produced by one object striking another, is reverberate and often is typed as a B-duration wave. On the other hand, blast waves, produced by rapidly expanding gases, have short durations and high peak levels often exceeding 150 dB. This class of impulse is an A-duration wave. Type A-waves have very brief rise times and generally include greater high-frequency energy as compared to Type B-waves that have longer rise-times and more low-frequency energy.

The acoustic transmission characteristics of impulse noises recorded from different locations within the uterus are not known with certainty. As more women of child-bearing age enter the military, concerns for the safety of the fetus of those women who become pregnant increases. Thus, a carefully controlled study of intraabdominal transmission characteristics of impulse noise was completed in four non-pregnant sheep exposed to 169 dB pSPL stimuli.

These adult ewes were killed as part of other experiments. Wool was sheared over the abdominal and flank areas and along a 4 in. swath running the full length of the spinal column. Ewes were then suspended horizontally from a metal frame according to the following procedures. Two punctures were made through the skin 2 in. apart at 7 locations over the spine from coccyx to occiput. A neoprene coated, 18 gauge copper wire was inserted through one wound and tunneled 5 cm under the skin where it was brought out through the second wound.

The seven wires were tied around the metal frame in such a way that the spine was parallel to the frame.

The frame and animal were then carried to the sound-treated booth, positioned so that the left flank of the ewe was 4 feet from the exponential horn that was attached to the shock tube. The exponential horn matched the impedance at the end of the shock tube with the impedance inside the booth. Impulse noises were created by the rupture of a mylar diaphragm with a pressure build-up of 70 psi. of nitrogen. A miniature hydrophone (Bruel & Kjaer Instruments, Inc. [B&K], Naerum, Denmark, model 8103) was used to record the SPL of the impulse in air. Simultaneously, a second hydrophone recorded the impulse in the abdominal cavity of the ewe.

The hydrophone was inserted into the abdominal cavity through an incision in the right mid-flank region (side of animal away from the horn). The hydrophone was pushed parallel to the floor of the booth until it was felt against the internal wall of the left flank (side of the animal nearest the horn). Sound pressure levels were recorded from this "proximal" position.

The hydrophone was then withdrawn along the axis of stimulation in order to place it midway between the proximal and distal (the point of entry on the right flank) positions, that is, deep in the abdomen. After recording from this position, the hydrophone was withdrawn so that it was just inside the abdominal cavity (distal position) where final measurements of the pSPL were completed.

The outputs from the hydrophones were fed into the two channels of a frequency analyzer that used constant percentage bandwidth filters (B&K, model 2123). Time waveforms were recorded simultaneously from the hydrophone positioned in line with the shock tube and directly above the proximal flank. The analyzer was set to trigger off of the lead edge of the waveform recorded in air. The second hydrophone was in the abdominal cavity in one of the three locations described above (Proximal, Medial or Distal). The time waveforms were saved to disk for off-line analysis of peak SPL, rise time, and peak duration. The waveforms were transformed into the frequency domain for analysis of the frequency content expressed in 1/3-octave bands.

Repeated measures analysis of variance (ANOVA), with site considered as a 3-level within-subject factor and the average of three replications considered as the response for each animal at each site, was used to test for an overall difference among response means (pSPL, rise-time, duration). Tukey's multiple pairwise comparison procedures were used to maintain a significance level of .05 for pairwise comparisons among animals and sites.

Peak sound pressure level was defined as the highest pressure in dB at the onset of the impulse (Coles, 1968). Rise-time was the time taken for the single pressure fluctuation that formed the initial positive peak to increase from ambient to the peak pressure level. Duration was calculated in two ways depending upon wave type. For A-duration pressure waves, duration was the time required for the initial pressure wave to rise to its positive peak and return momentarily to ambient. For B-duration pressure waves, duration was the time interval between impulse onset and the point in time at which the envelope decayed by 10 dB from peak level (Smoorenburg, 1992).

Table I includes the average values from the four animals for pSPL, rise-time and duration. A judgment regarding the wave type is also included. In the proximal location, an A-duration pressure wave was noted in three of the four animals. In the fourth ewe, a type B-duration pressure wave was observed. The morphology of the pressure waves recorded in air

was very similar to the morphology recorded at the proximal location, yet was distinctly different than the waveforms recorded at the medial and distal locations.

Table I

Location	PSPL	Type	Rise-time (ms)	Duration (ms)
Air	169.4	4-A	0.12	0.82
Proximal	165.7	3-A; 1-B	0.44	6.67
Medial	150.9	4-B	2.47	41.47
Distal	154.7	4-B	1.22	36.41

The ANOVA applied to pSPL values revealed significant differences as a function of hydrophone location ($F_{3,9}$=40.19; p=<.0001). The Tukey multiple pairwise comparison showed that the pSPL for air (169.4 dB) was essentially the same as that for the proximal location (165.7 dB), yet significantly different than the medial (150.9 dB) and distal (154.7 dB) values.

Evaluation of values for rise-time and duration revealed a similar pattern of results. Statistically significant main effects were noted for both analyses. Post hoc testing indicated that values for rise-time and duration were not different when recorded in air and at the proximal location. However, rise-time and duration recordings differed statistically between air recordings and those made at either the medial or distal locations.

All pressure waveforms were transformed into the frequency domain and averaged as a function of recording location. Figure 5 displays the results of this process in 1/3-octave bands. A few observations warrant comment. First, the 1/3 octave band levels from 200-315 Hz are greater when recorded at the proximal location as compared to air. Enhancement of low-frequency sound pressure within the abdomen has been observed in other studies (Gerhardt et al., 1990; Querleu et al., 1981). The tendency for low-frequency enhancement to occur may relate to intraabdominal resonance.

Second, there is a noticeable drop in level above 500 Hz when comparing air to the proximal recording site. This drop, or attenuation, exceeds 10 dB at 5000 Hz and has been noted for steady-state noise and tones (Gerhardt, et al., 1990; Querleu et al., 1988). Considerably greater attenuation for the high frequencies is seen for recordings at the medial and distal locations. Differences of 20-30 dB are seen in this figure between air and recordings at the medial location.

The data from this study are being prepared for submission to Military Medicine. We expect that the manuscript will be ready in six months.

Evoked Potential Response Thresholds. Auditory brainstem response (ABR) and amplitude modulation following response (AMFR) thresholds were measured prior to and immediately following the exposure to 20 impulses produced by the shock tube at either 117 days gestational age (in the early-exposed group), or at 127 days gestational age (in the late-exposed group). Subsequent measurements of ABR and AMFR thresholds were obtained every two to three days for the duration of the experiment in all animals in both of the groups. Analyses were undertaken to examine the effects of time of measurement (i.e., pre-exposure,

post-exposure, and the subsequent recovery measurements) and stimulus type (i.e., clicks and tone-bursts for the ABR and 500 and 1000 Hz amplitude-modulated tones for the AMFR).

Figure 6 contains the averaged thresholds obtained from the early-exposed group of animals across time for the ABR in response to clicks and tone bursts, and for the AMFR in response to 500 and 1000 Hz carrier frequencies. One can note several effects in this figure. For all stimuli there is an obvious developmental trend toward lower thresholds at later gestational ages. Developmental changes in ABR thresholds in fetal sheep have been reported earlier by Pierson et al., 1995. In addition, a small elevation in thresholds may be noted to disrupt the progressive drop in the post-exposure measure (the third bar from the left in each stimulus series) for the Click- and 2000-Hz ABR, and the 500 Hz AMFR conditions. The 4000 Hz ABR thresholds reveal no obvious influence of the exposure to the impulses. Statistical analysis using analysis of variance (ANOVA) yielded significant effects for stimulus frequency ($F = 19.843$, $p = 0.0001$) and time of measurement ($F = 1.884$, $p = 0.044$). Post hoc testing indicated that the pre-1 and post-exposure thresholds were significantly higher than those in the later measurement periods for the click, and 2.0 and 1.0 kHz stimuli.

Figure 7 contains the averaged thresholds obtained from the late-exposed group of animals across time for the ABR to clicks and tone bursts, and for the AMFR in response to 500 and 1000 Hz carrier frequencies. The developmental trends toward lower thresholds at later gestational ages seen in the early-exposed group are not apparent in the averaged thresholds in the late-exposed group. As in the preceding figure, the thresholds obtained immediately post-exposure are represented by the third bar from the left in the series for each stimulus type. An elevation in the 500 Hz ABR threshold of approximately 5 dB may be noted in Figure 7.

ABR Latencies. Latencies for wave IV (the most robust of the ovine ABR components, and the one which appears to correspond to wave V in the human ABR) were measured from all animals on all days at all stimulus levels where such measurements were possible. Latency measures were subjected to a two-way (time of measurement and stimulus frequency) repeated measures ANOVA. As might be predicted, stimulus frequency significantly affected wave IV latencies with clicks and higher frequency tone bursts producing the shortest latencies.

ABR wave latencies in the early-exposed group were also significantly affected by the time of measurement ($F=13.038$, $p=0.0001$). Post-hoc testing revealed significant differences between the latencies obtained during the first four measurement periods (Pre-1, Pre-2, Post, and Recov-1) and those obtained in the subsequent periods. This finding was noted for all ABR stimulus conditions (click, 4.0, 2.0 and 1.0 kHz tone bursts). Figure 8 displays average Wave IV latencies as a function of time of measurement for clicks and 4.0, 2.0 and 1.0 kHz tone bursts delivered at 60 dB nHL. One may note the developmental trend toward shorter latencies at later gestational ages. This consistent pattern appears to be temporarily interrupted following the exposure to the blasts, as the post-exposure latencies are equal to or greater than the immediate pre-exposure values.

In the more auditorily mature, late-exposed group, ABR latencies did not show the same developmental changes. Figure 9 displays average wave IV latencies in response to clicks and tone bursts at 4.0, 2.0 and 1.0 kHz delivered at 60 dB nHL as a function of measurement period in the late-exposed group. One may note the absence of any demonstrable change from the pre-

exposure to the post-exposure measures, as well as the relative stability of ABR latency across the later gestational ages. This finding is borne out by the lack of any significant differences in latency (other than the effect of stimulus frequency) from the ANOVA.

Histology. The temporal bones were removed bilaterally and fixed with phosphate-buffered 2.5% glutaraldehyde and 2% paraformaldehyde mixture for 24 hours, and then decalcified with 15% EDTA (phosphate-buffered) for 10 days. Subsequently, the temporal bones were washed in phosphate-buffered solution, post-fixed in a 1% osmium tetroxide phosphate-buffered solution for one hour, and dehydrated in a graded series of ethanol up to 70% for micro-dissection. After dissection of the organ of Corti, the specimens were critical-point dried in 100% ethanol using liquid carbon dioxide, mounted on aluminum stubs, sputter coated with platinum to a depth of approximately 75 nm and examined with a field emission SEM. Serial photomicrographs of the entire length of the organ of Corti were used to construct a cochleogram for each cochlea.

Both the inner hair cells (IHC) and the outer hair cells (OHC) from undamaged regions of the organ of Corti had normal appearance with an orderly arrangement of stereocilia except for the apical region closest to helicotrema. The shape of OHC stereocilia resembled the classic "W" pattern, the IHC stereocilia resembled a "U", and the length of stereocilia decreased from apex to base as seen in other mammals (Gulick et al., 1989).

Following noise exposure, hair cell damage of both IHC and OHC was noted primarily in apical and middle turns of the cochlea. Abnormalities of hair cells included distorted and/or missing stereocilia, giant stereocilia and phalangeal scarring. Stereocilia of the IHC were often missing in the apical turn. Stereocilia of the OHC were missing, distorted, and in some cases broken apart. Also, giant stereocilia were observed in the apical turn. Although in some instances, stereocilia of IHC and OHC were relatively intact, cytoplasm ballooned out of the hair cells and at junctions of supportive cells (Figure 10). Ballooning of cytoplasm has been reported to arise from the apical end of the hair cells following noise exposure (Hunter-Duvar et al., 1982). In the middle turn, there was evidence also of phalangeal scarring, yet fewer distorted stereocilia were noted as compared to the apical turn (Figure 11). The IHC and OHC in the basal turn appeared intact with no evidence of cytoplasmic ballooning, phalangeal scarring or missing, distorted and broken stereocilia.

There appears to be a progression of change to the hair cells that ultimately ends with complete replacement of sensory epithelium by surrounding supporting cells. In some instances, a webbing formed over the tops of damaged stereocilia (Figure 12). Following what appeared to be a fairly rapid process, the stereocilia were absorbed and the webbing ended up lying flat on the apical end of the hair cell (Figure 13). The hair cell itself was completely absorbed and replaced with supporting cells, presumably Deiter cells (Figure 14).

Cochleograms were plotted for each cochlea following procedures described by Schuknecht (1953). Damaged or distorted stereocilia and presumed locations of missing hair cells were counted and mapped by location. Stereocilia were considered damaged if they were absent or altered in any way. Areas of damage are indicated as a percentage of hair cell loss as a function of distance along the organ of Corti. The individual cochleograms, as a function of gestational age at the time of the exposure, were averaged and plotted as mean cochleograms

(Figure 15). Note that most hair cell damage found in the noise-exposed animals was confined to the middle and apical turns. On average, the IHCs were more severely affected than the OHCs, primarily in the apical region of the cochlear duct between 5 and 20% of the total distance from helicotrema.

Scanning electron micrographs shown in this study revealed damage consistent with reports of inner and outer hair cell alternations following noise exposure in other species (Hunter-Duvar et. Al., 1982; Saunders et al., 1985). When lesions occur in most smaller laboratory animals, OHCs are the first to be damaged and end up as the most severely altered when compared to IHCs. In contrast, fetal sheep demonstrated greater damage on average to IHCs than OHCs. While not observed in most experimental animals, greater damage to IHCs than OHCs following noise exposure has been reported in rabbits (Engström and Borg, 1981). Evaluation of cellular damage from noise-exposed fetal sheep revealed a greater loss of OHC3 as compared to the other rows of OHCs. A similar finding has been reported in adult monkeys (Moody et al., 1978).

The electrophysiological and histological data are currently being organized for publication in the Journal of the Acoustical Society of America. We anticipate submitting the manuscript within the next nine months.

Fetal Behavioral State. Data collection has been completed on behavioral state and heart rate from 10 fetuses. Measurements were obtained from the fetuses exposed to noise at 127 days gestational age. Behavioral state does not develop in the fetus until after about 120 days gestational age and therefore the fetuses exposed at 117 days gestational age were not evaluated. The strip chart recordings and magnetic tapes have been sent to colleagues in Jena, Germany where complete analyses are underway of spectral changes in electrocortical (ECoG) activity and heart rate responses before, during and after impulse exposure. Procedures follow techniques reported by Bauer et al., 1997.

Results of animal O-105 are fairly representative of these ten animals and demonstrate a marked affect of the impulses on behavioral state. However, considerable individual variation was noted. The analysis followed the procedures described by Szeto and Hinman, 1985. Twenty impulses delivered to the ewe spaced about one-minute apart resulted in increased alpha and theta activity during and slightly after stimulation, whereas, delta activity remained low. At the beginning of stimulation, the ECoG amplitude dropped as did fetal eye movement amplitude.

The amplitude of a period of REM sleep was compared to brief periods of ECoG activity recorded at 5, 15 and 30 sec after each of the 20 impulses. Figure 16 shows the results of these comparisons. The upper panel in this figure reveals that 5 sec after the blast, the amplitude of the ECoG consistently exceeded the amplitude of the ECoG recorded during a control period of REM. This same finding was noted 15 and 30 sec after the blasts. Results from this animal suggest a fairly strong effect of the impulses on fetal behavioral state at least during the time-period over which our recordings were made. We have no evidence that behavioral state is disrupted for longer periods of time after cessation of impulse exposure.

In other animals, little effect of the impulse exposure was noted on ECoG activity and in some cases no change in behavioral state at all was found. These data are currently being organized for publication within the next six months.

Recommendations Related to Statement of Work

Below is a summary of the tasks listed in the Statement of Work:

Tasks Proposed:	Status:
Order ABR and AMFR recording equipment	Completed
Construct shock-tube	Completed
Test shock tube (air measurements)	Completed
Confirm shock tube signature in water	Completed
Collect data on ten animals (Study 1)	Completed
Submit annual Report	Completed
Collect data on 20 animals (Study 2)	Completed
Evaluate histopathology	Completed
Final Report to U.S. Army	Completed

Data analysis was more time-consuming than originally planned. We were forced to adjust to a tight schedule in order to gain access to the new scanning electron microscope in the University's Core Microscope Center, so this portion of the project has taken more time than originally planned. However, all cochlear tissues have been prepared and micrographs completed. Cochleograms have been plotted and included in average figures. We are in the process of preparing three manuscripts for publication and expect to have then submitted within the next 6-9 months.

CONCLUSIONS

Peak SPLs recorded from within the uterus were highly variable and ranged from 153 to 168 dB. Peak levels in air averaged 169.9 dB. The overall morphology of the waveforms related to pSPL and frequency content of the impulse. Peak levels recorded in the uterus averaged 7.3 dB less than those recorded in air. Spectral analysis in one-third octave-bands revealed peak levels in air at 315 Hz compared to 160 Hz when recorded from the uterus. High-frequency sound pressures were attenuated by the tissues and fluids of the ewe by up to 25 dB, as predicted from earlier studies.

The position of the hydrophone within the uterus influenced both pSPL as well as spectral distribution. When the hydrophone was near the abdominal surface, peak levels were approximately 2 dB less than the peak levels recorded in air. When the hydrophone was deep within the uterus, the morphology of the waveform changed and peak levels were approximately 20 dB less than those recorded in air.

Electrophysiologic thresholds were examined over time. Small elevations in the mean thresholds for the 0.5 kHz stimuli (ABR) were noted in the post-exposure measures. Thresholds improved in recordings over the next 10 days. No similar elevations were noted for the higher-frequency tone bursts or clicks, or for the AMFR. Similarly, continuous, intense broadband noise exposures delivered to fetal sheep resulted in temporary post-exposure threshold elevations for low-frequency tone bursts (Griffiths et al., 1994). ABR latencies shortened as gestational age increased in the early noised-exposed fetuses. Exposure to impulses did not affect ABR latencies in either group of fetuses.

Cochleae from the fetuses were examined using scanning electron microscopy. Hair cells from noise-exposed fetuses appeared different in a number of respects from historical control fetuses from this laboratory (Gerhardt et al., In Press). Damage to both inner and outer hair cells was noted primarily in the apical and middle turns of the cochlea. Abnormalities included bent and/or missing stereocilia, giant stereocilia and phalangeal scarring. The damage found in fetal sheep inner ears is consistent with reports following noise exposures in other species (Saunders et al., 1985). Inner hair cells were more severely affected than outer hair cells in the apical region between 5 and 20% of the total distance from helicotrema. These findings are quite different than would be predicted based upon knowledge of adult noise-induced hearing loss that affects the basal region of the cochlea.

The findings that noise exposure to the fetus *in utero* affect inner ear histology apply only to fetal sheep. There are no compelling data to demonstrate that human fetuses have the same susceptibility to noise as do sheep, or that human fetuses are at risk to inner ear damage produced by noise levels to which pregnant women might normally be exposed. However, the data from this study warrant consideration in the formulation of guidelines that may be developed to protect the fetus of pregnant women from noise damage.

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APPENDIX

Figures 1 through 16.

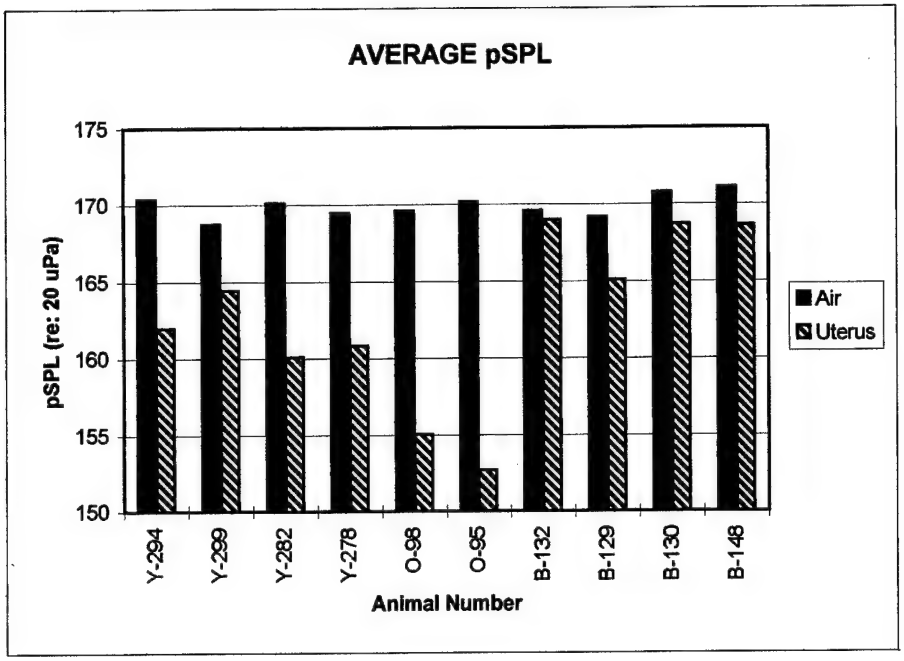

Figure 1. Average peak sound pressure level (pSPL) of 20 impulses per animal recorded simultaneously in air and in the uterus. The averages of all impulses were 169.9 dB (S.D.=.92) in air and 162.6 dB (S.D.= 5.94) in the uterus.

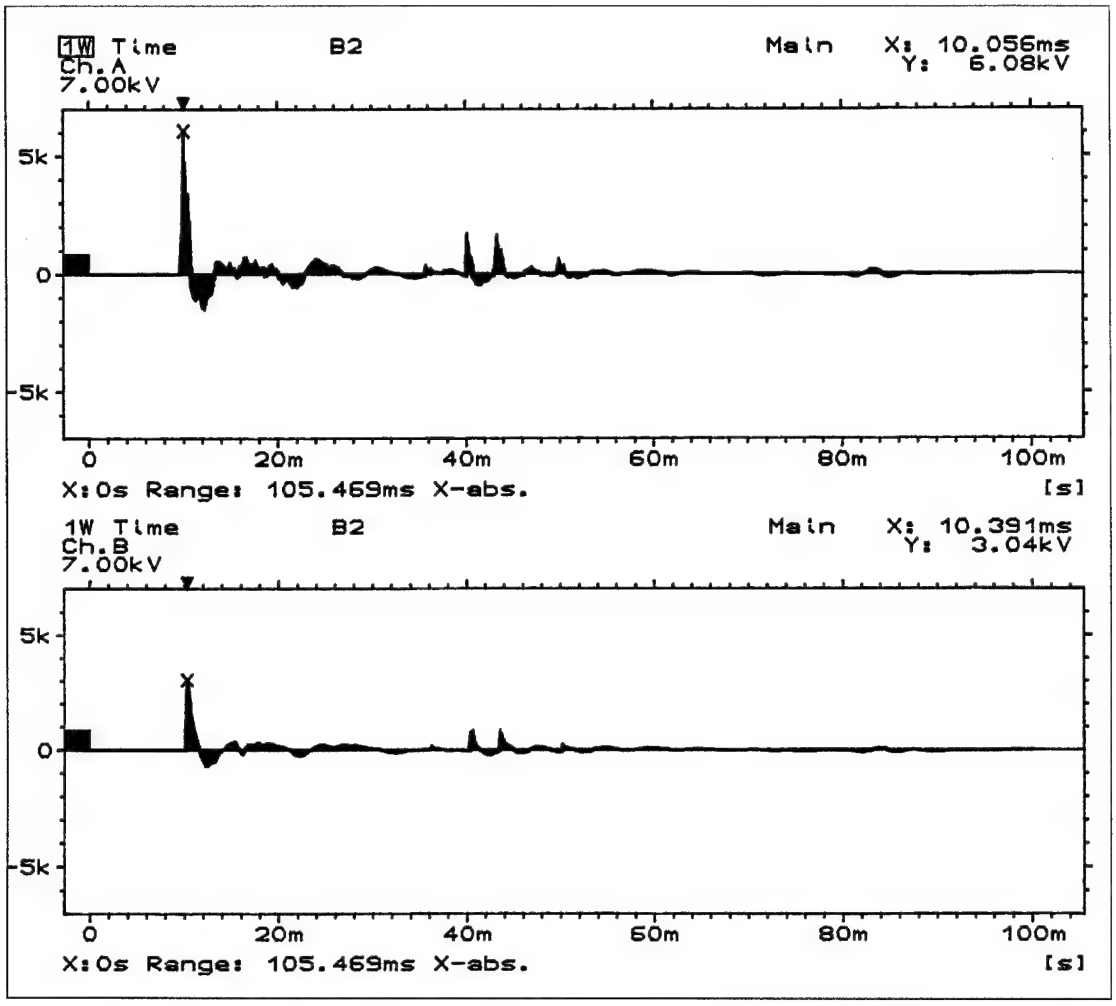

Figure 2. Waveforms of an impulse recorded simultaneously with hydrophones in air (upper plate) and in the uterus of a pregnant sheep (lower plate). The hydrophone in the uterus was within 1 inch of the flank nearest the shock tube. The peak sound pressure levels were 169 dB and 166 dB in air and in the uterus, respectively.

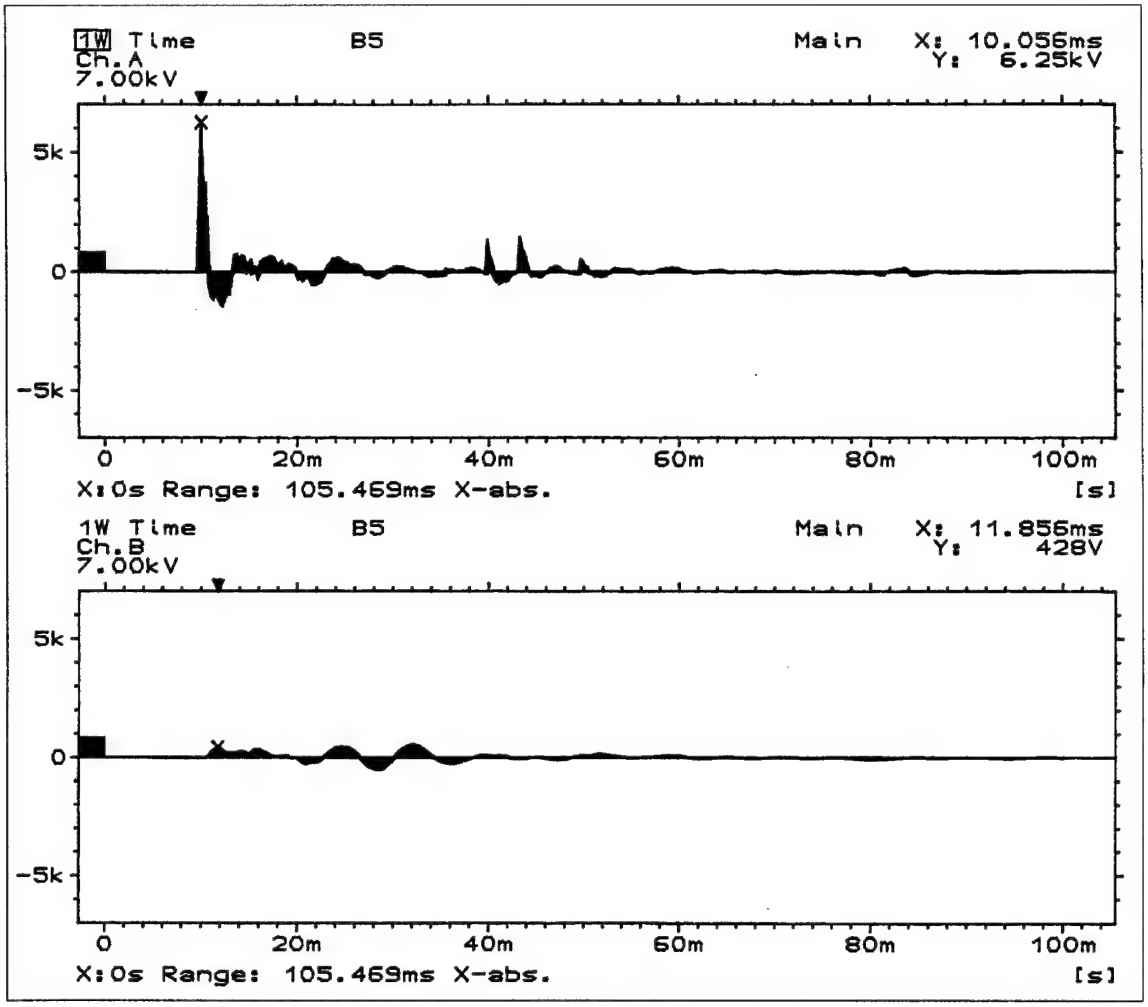

Figure 3. Waveforms of an impulse recorded simultaneously with hydrophones in air (upper plate) and in the uterus of a pregnant sheep (lower plate). The hydrophone was deep within in the uterus at midline. The peak sound pressure levels were 169 dB and 147 dB in air and in the uterus, respectively.

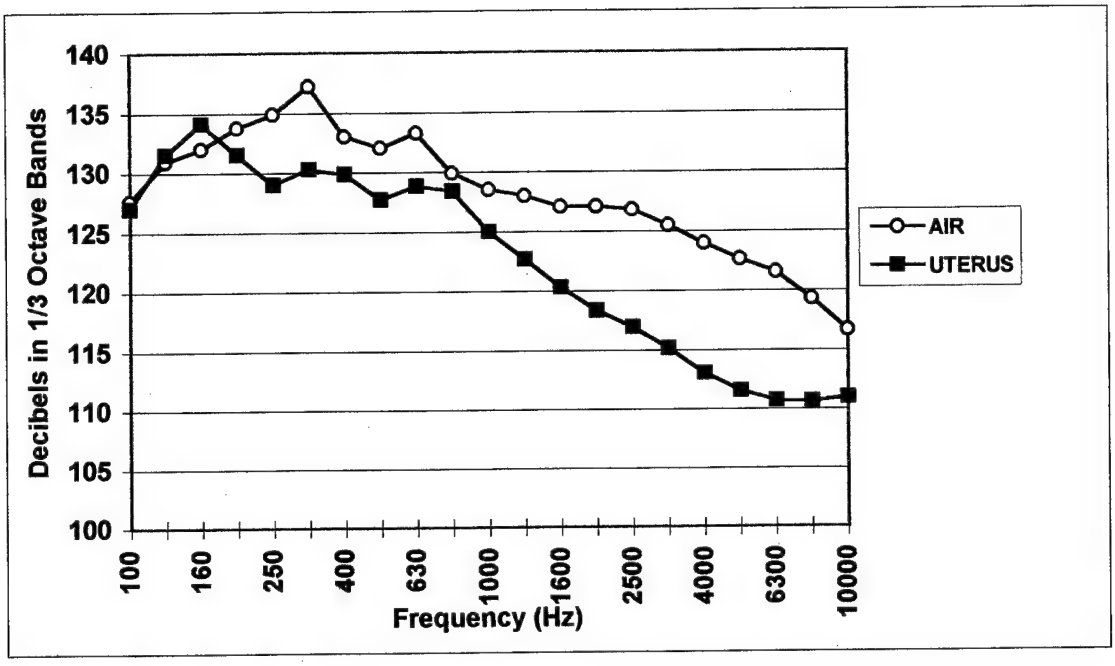

Figure 4. Average spectra of impulses recorded simultaneously in air and in the uterus of pregnant sheep.

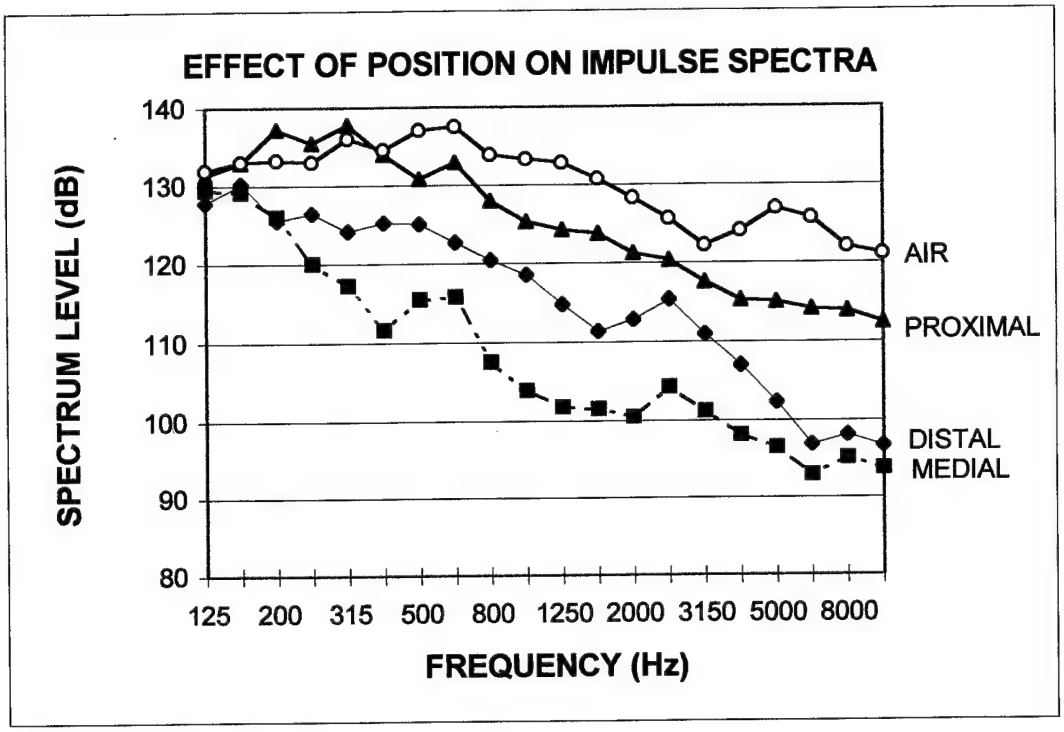

Figure 5. Spectra of impulses recorded with hydrophones in air and positioned at three locations within the abdomen of non-pregnant sheep. Proximal- the hydrophone was within one inch of the surface of the flank closest to the shock tube. Medial- the hydrophone was positioned at the midline of the abdomen. Distal- the hydrophone was within one inch of the surface of the flank furtherest from the shock tube.

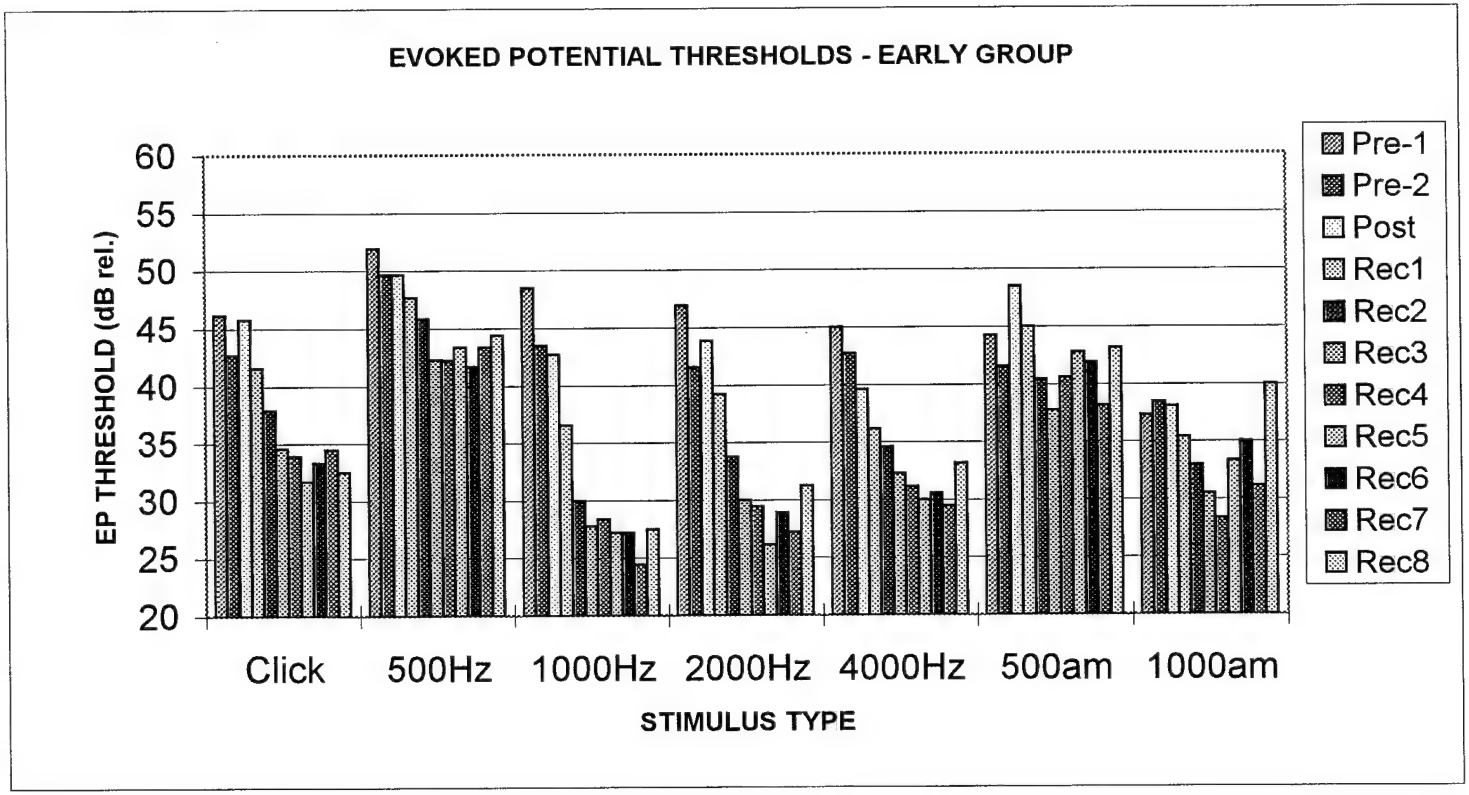

Figure 6: Evoked potential thresholds in the early-exposed group of animals displayed as a function of stimulus type and measurement period.

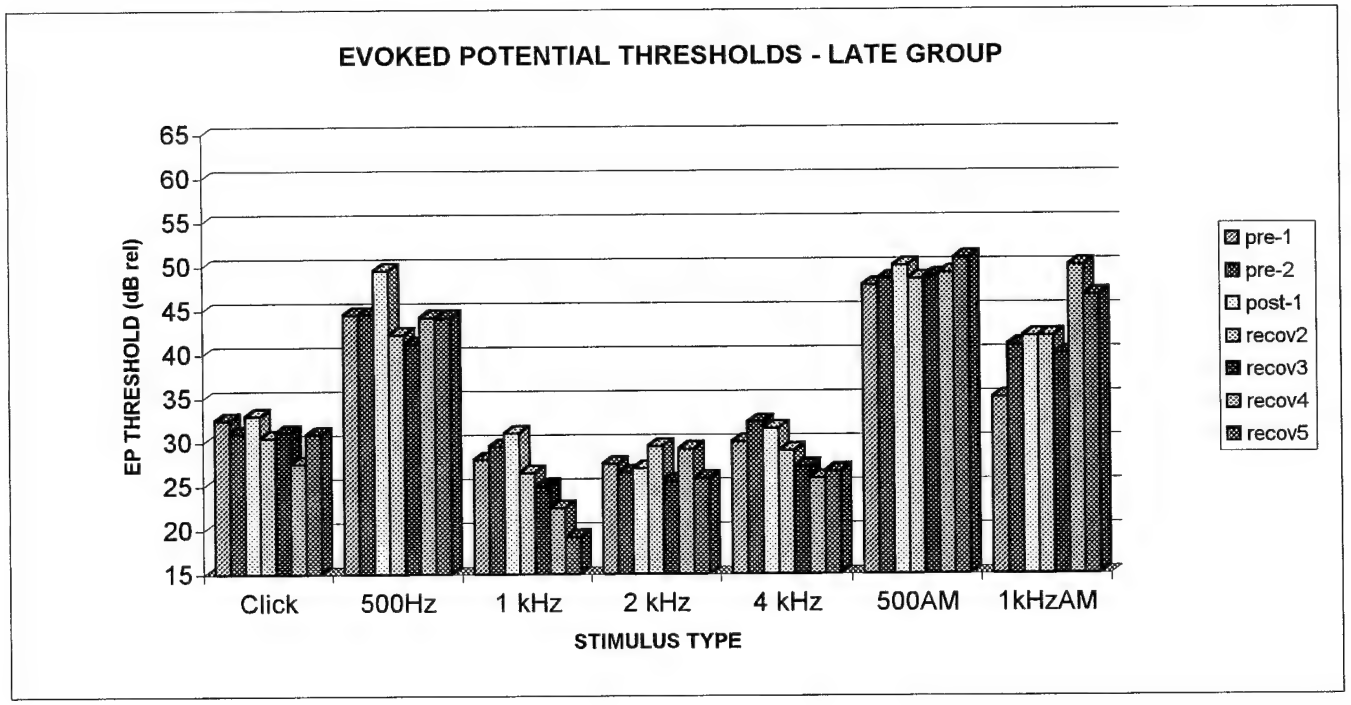

Figure 7: Evoked potential thresholds in the late-exposed group of animals displayed as a function of stimulus type and measurement period.

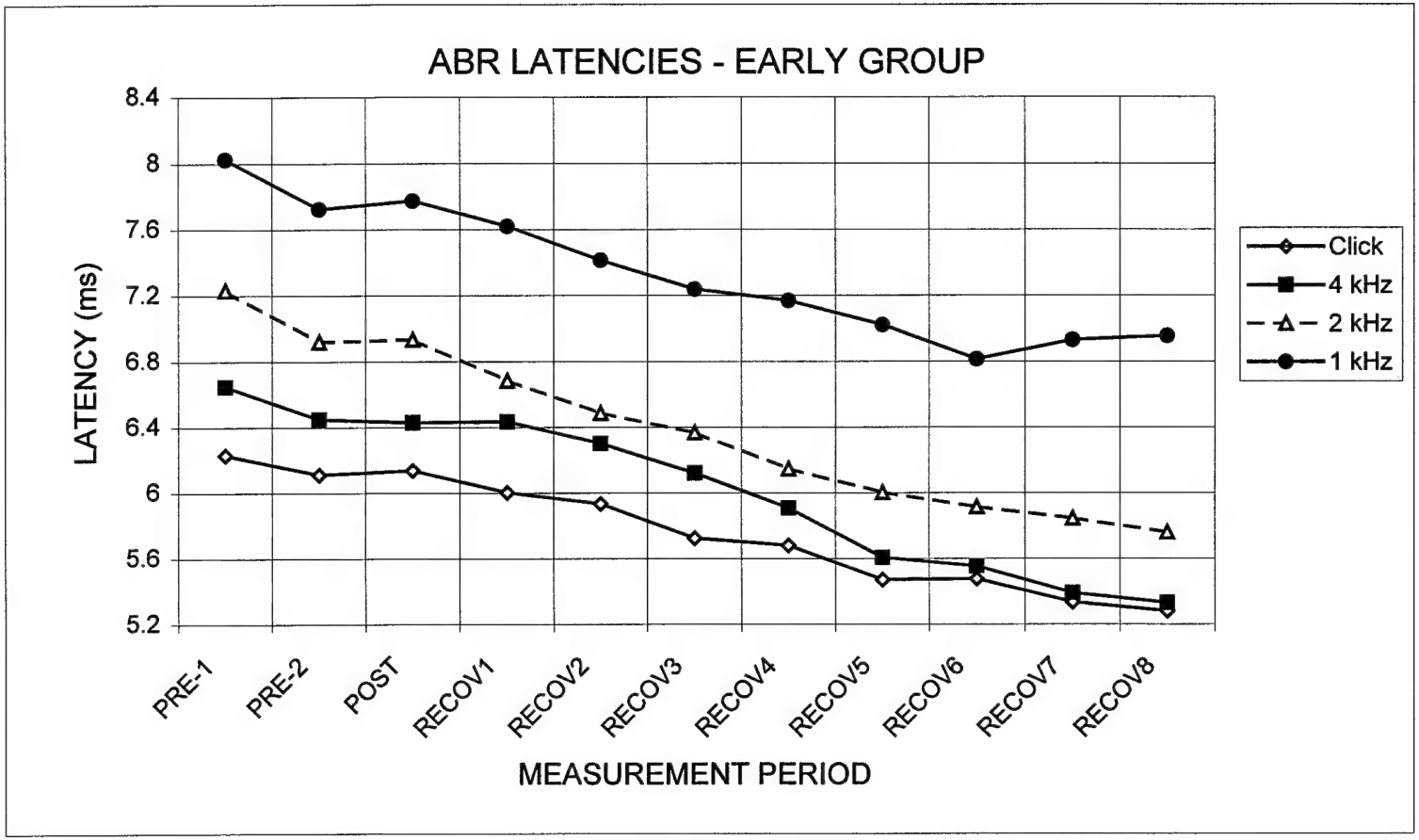

Figure 8: Auditory brainstem response latencies in the early-exposed group of animals displayed as a function of measurement period and stimulus type (clicks and 4.0, 2.0 and 1.0 kHz tone bursts delivered at 60 dB rel.).

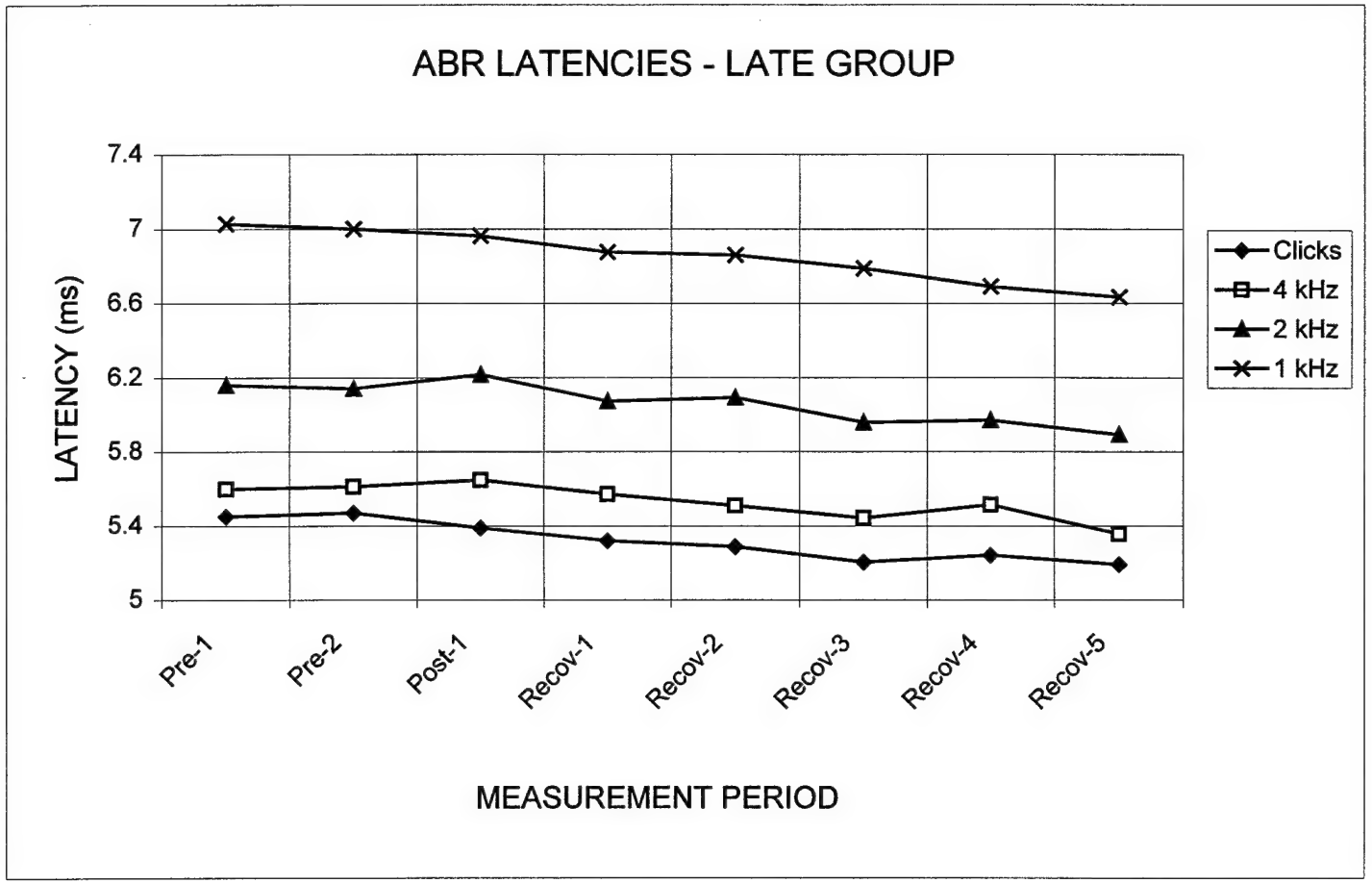

Figure 9: Auditory brainstem response latencies in the late-exposed group of animals displayed as a function of measurement period and stimulus type (clicks and 4.0, 2.0 and 1.0 kHz tone bursts delivered at 60 dB rel).

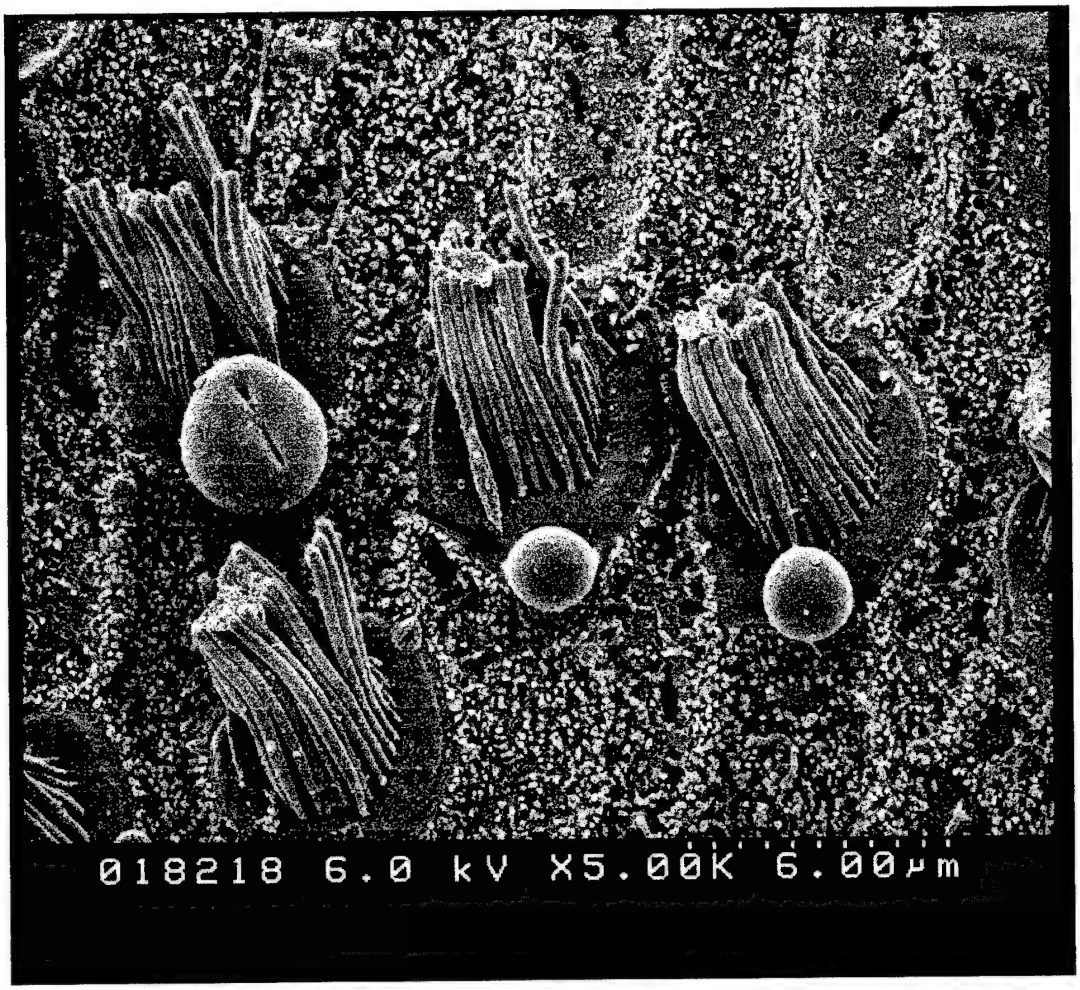

Figure 10. Arrows indicate cytoplasmic ballooning of outer hair cells from the apical portion of the cochlea of a noise-exposed fetal sheep.

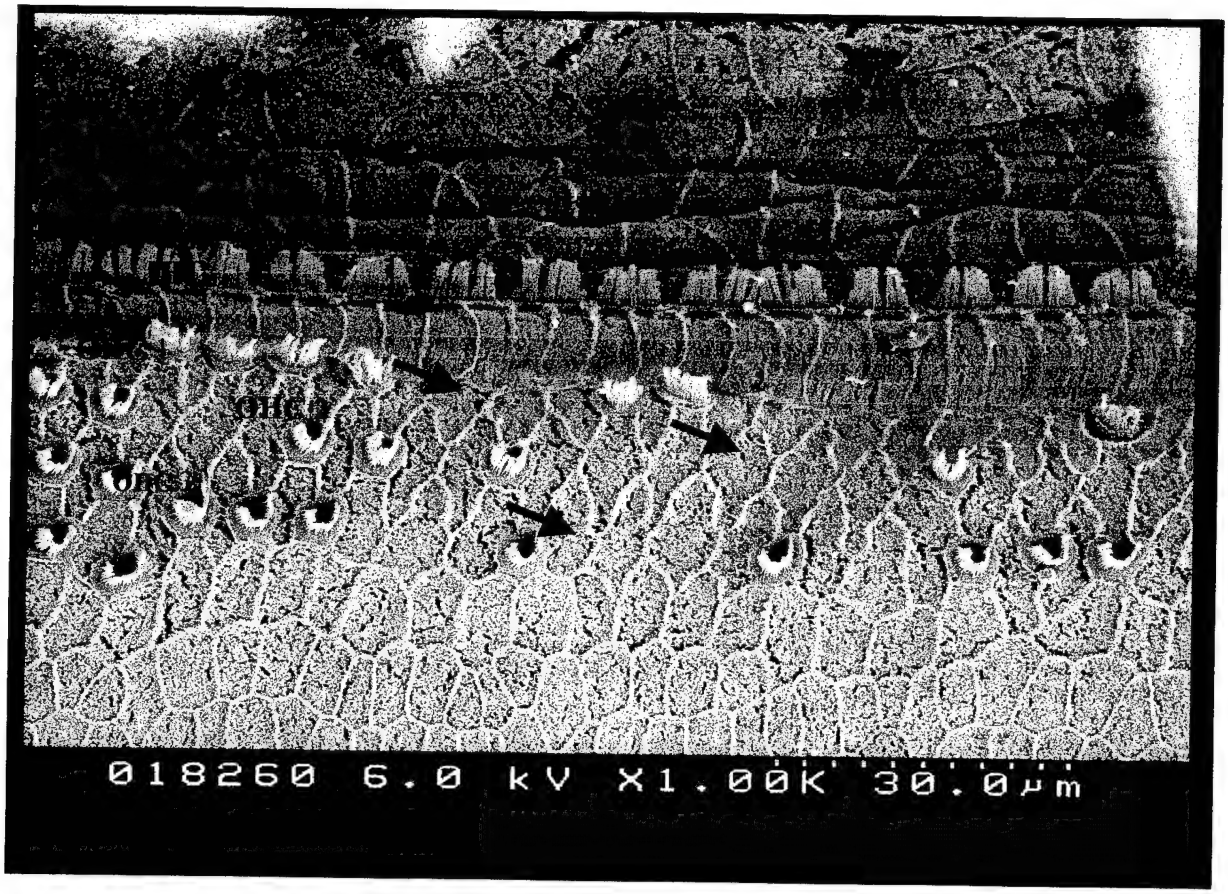

Figure 11. Arrows indicate locations of missing outer hair cell (OHC) in all three rows of the middle turn of a noise-exposed fetal sheep. Phalangeal scars have completely replaced the OHC.

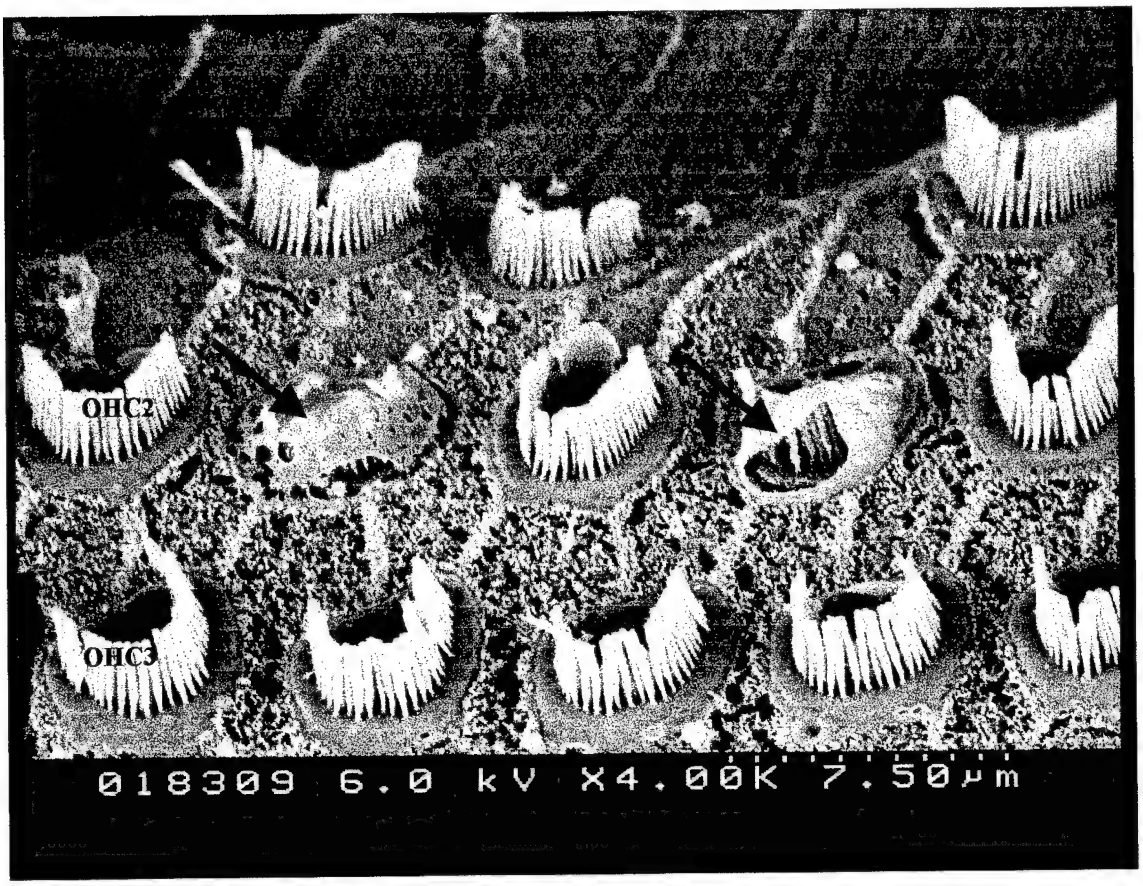

Figure 12. Arrows indicate a webbing-like material that has formed over the stereocilia of OHC2 of noise-exposed fetal sheep.

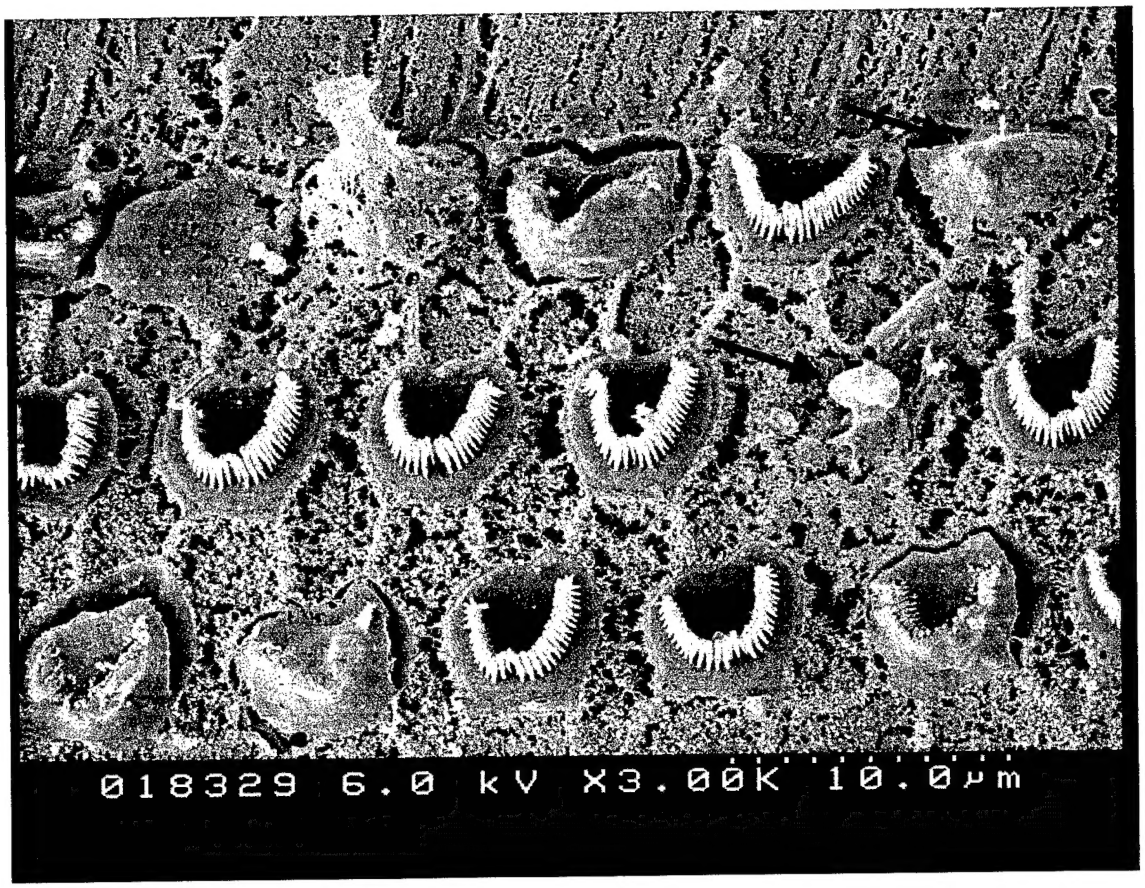

Figure 13. Stereocilia under the webbing-like material are almost completely dissolved (Arrows).

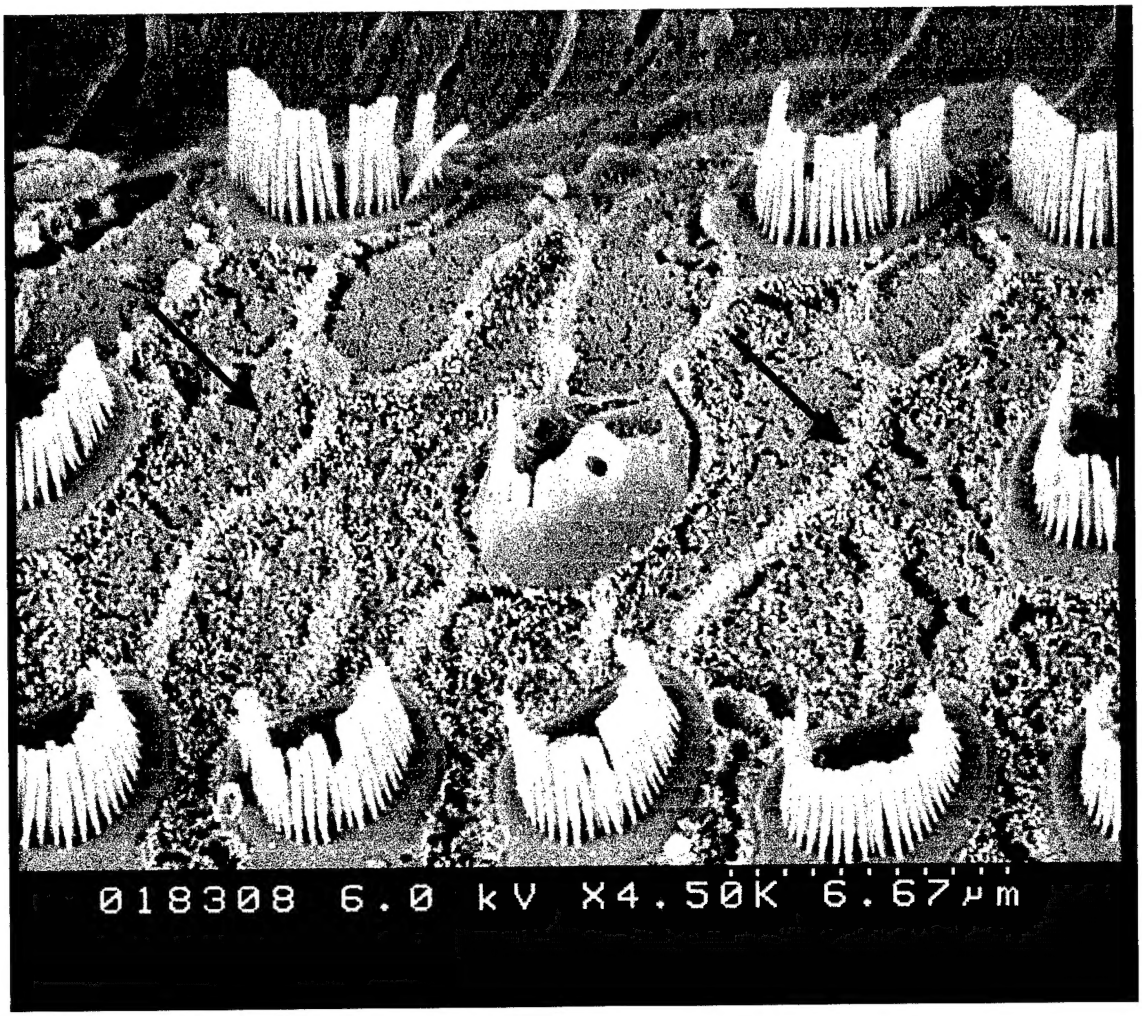

Figure 14. Hair cells in OHC2 are completely gone and have been replaced with phalangeal tissue (Arrows).

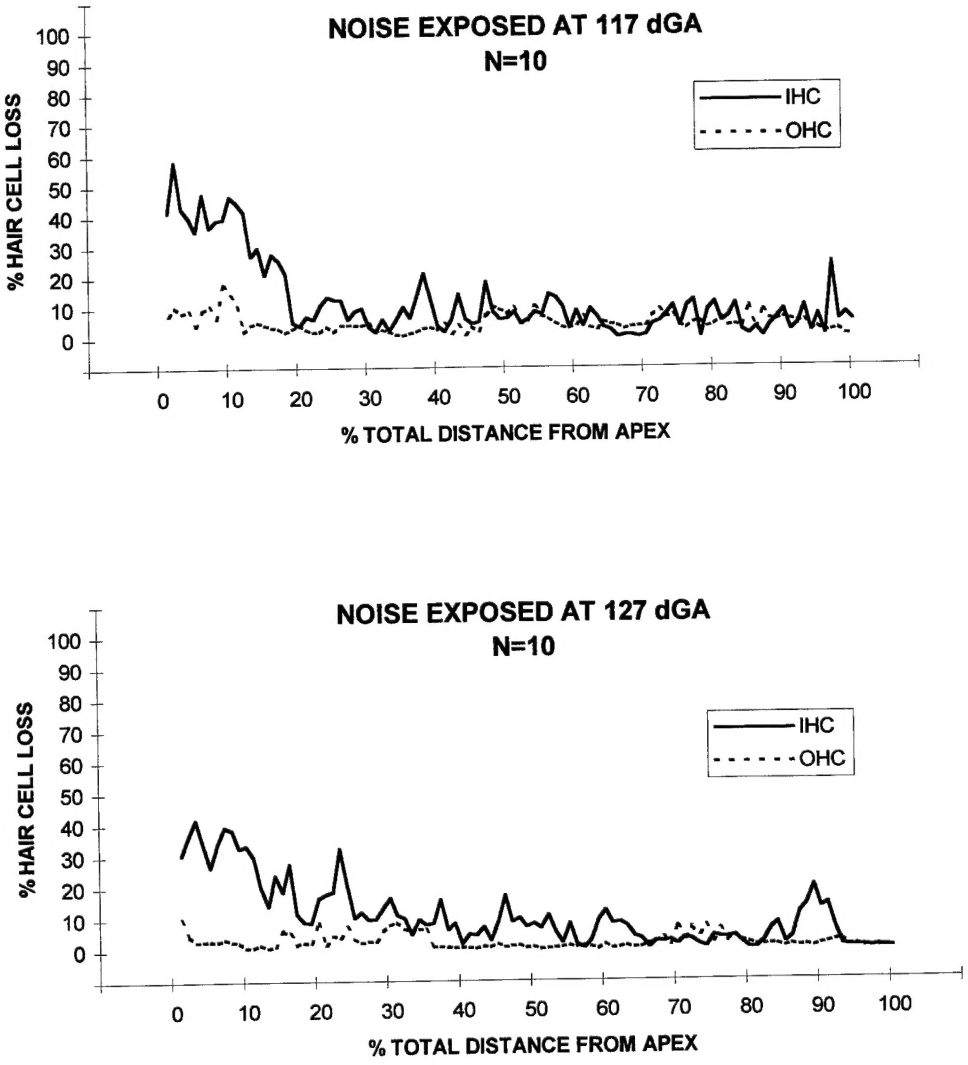

Figure 15. Average percentage of damage to inner (IHC) and outer hair cells (OHC) of fetal sheep cochleae. The cochleograms show hair cell integrity from fetuses exposed at 117 and 127 days gestational age (dGA) to 20 impulses at 169 dB peak sound pressure level.

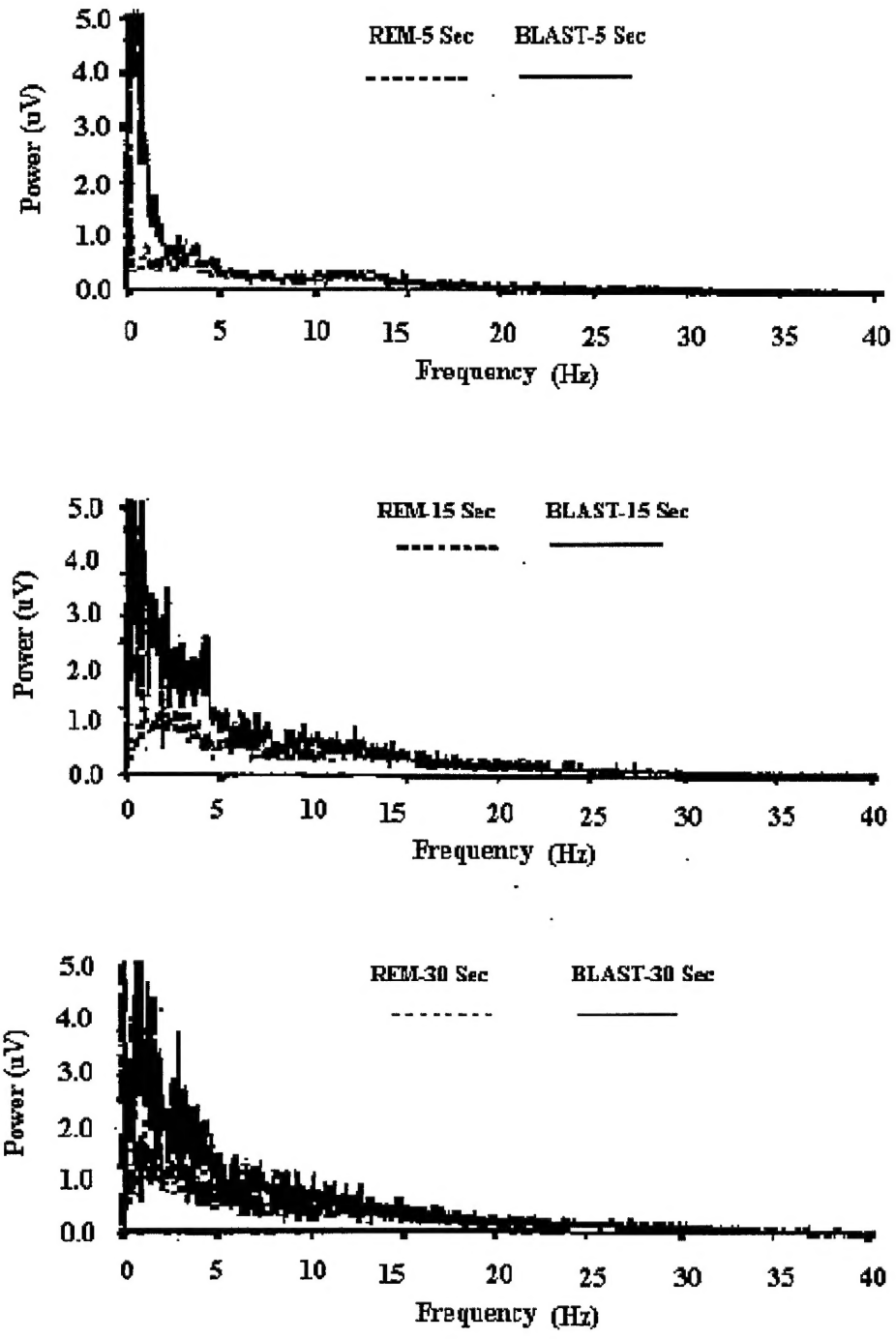

Figure 16. Spectra of fetal sheep rapid-eye movement (REM) sleep recorded before and during exposure to 20 impulses. REM was recorded 5, 15 and 30 seconds after each of 20 blasts.